Where's the CAT Simulation

Scenario
A woman was jogging late at night when she was attacked and raped in the park. The woman was immediately taken to the hospital where a rape kit was done, and DNA was collected. Meanwhile, surveillance videos at the park were collected allowing police to narrow the suspect field to two men. Your task will be to analyze the DNA from the crime scene, the DNA from each suspect, and the DNA from the victim. You will then compare each sample to determine which of the suspects is the likely rapist. Keep in mind that the DNA sample collected from the victim will likely contain both the victim and the rapist's DNA so you should not expect to find a perfect match due to contamination.

Background
DNA profiling (also called DNA fingerprinting) is now being used in some criminal and legal cases where DNA samples are available to determine identity or parentage. DNA may be extracted from relatively small samples of cells, such as a blood stain the size of a nickel (about two drops) or a body fluid stain the size of a dime. When performed under properly controlled conditions and interpreted by an experienced forensic scientist, such profiling can link a suspect to a particular incident with compelling accuracy or completely exonerate a suspect. At the DNA level individual people are about 99.9% identical; they differ on average in 1 out of 1000 base pairs. Some of these differences are in genes which lead to the visible differences between us. Some of the differences, however, are in ''junk'' DNA (DNA which as far as is known is not transcribed into RNA). In 1984, Sir Alec Jeffrey's discovered short nucleotide sequences (3 to 30 base pairs in length) that were repeated multiple times (10 to 100 times) in non-coding regions of DNA. These are known as Variable Number Tandem Repeats, or VNTRs. In each case, what is variable is the number of copies of the sequence in an allele. So for example, if the repeated sequence were CAT, in one allele there might be 3 copies [CATCATCAT] whereas another allele might have 7 copies [CATCATCATCATCATCATCAT]. In a given population there may be dozens or even hundreds of different VNTR alleles. Of course, any individual has only two alleles, one on each of the homologous chromosomes (one each of which was inherited from one parent). Since there are so many alleles in a population, most people are heterozygous for alleles of any given VNTR. If one examines enough different VNTRs (6 to 12) in a given person, one can put together a molecular picture or ''DNA fingerprint'' of that person. This can be used for identification of tissue left at the scene of a crime (body fluid from an assault victim) or for paternity testing, in which case the VNTR alleles in the child that are not present in the mother must have come from the biological father.

Materials
o Large sheet of paper or poster board for gel (at least 60 cm x 80 cm)
o scissors and tape (or glue)
o set of base sequences representing the ''Standards,'' ''Mother DNA,'' ''Child DNA,'' o ''Suspect DNA,'' and ''Husband DNA''
o set of ''Probe'' sequences copied onto brightly colored paper

Procedure
1. Cut out the strips of DNA sequences for each of the individuals and the standards. Be sure to keep each individual's DNA strands separate from each other.
2. We will be using the restriction endonuclease Hind III. Mark the sample strip at the recognition sites for the restriction enzyme (AAGCTT) and cut the strip all the way across between the two A's of each restriction site.
3. Use the desktop or a large sheet of newsprint to simulate the gel electrophoresis apparatus. The standards should be placed first. Use the top of the desk or paper to represent the wells of the gel. Exact distances from the origin in the ''well'' are not important, as long as all fragments of the same length are placed the same distance from the well. The larger fragments are placed closest to the well with the

smaller ones being placed further away in descending order beneath the well. The standards should span almost the whole distance, leaving perhaps 5 cm at the bottom.

4. Place the fragments of the mother's sample to the right of the standard sample well. Note that the mother's 12-base fragment should be the same distance from its well as the standard 12-base fragment is from its well.
5. Continue placement of the remaining samples in the same manner, moving to the right across the paper or desk top in the following order: husband, suspect, and child. When complete, each sample should contain 5 different fragments.
6. On a separate sheet of paper, sketch the results of this electrophoresis event. Remember that in ''real life'' these fragments would be invisible to the naked eye.
7. These fragments must next be differentiated from one another by use of the ''probe.'' Construct DNA probes by cutting the simulated fluorescent probes from the brightly colored paper containing the probe sequences. These DNA probes will be used to see (''visualize'' as the scientists call it) the VNTR sequences. (Remember that the probe sequence, 3'GTAGTA5', is complementary to the VNTR sequences, 5'CATCAT3'. With a probe in hand, scan the ''gel'' and position a probe on each complementary sequence. Each labeled fragment represents a part of one chromosome of a homologous pair.
8. On your previous sketch of the unmarked gel, identify the fluorescently marked fragments by lightly coloring over them with a colored pencil: (Mom – pink, Husband – Blue, Suspect – Yellow, Child – Red).

Analysis

When you have completed "running your gel" and adding the radioactive probes, try to determine if the crime scene DNA evidence matches any of the other DNA samples, then:

- The band patterns and positions will be the same
- The radioactive probes will be aligned in the same location
- The number of VNTRs (repeats of CAT will be the same within each gene.

Questions

After completing your DNA gel and standard comparison answer each of the following questions

1. Which DNA samples match? Explain your answer

2. Explain why it was necessary to run a sample of the victim's own DNA. Explain how the DNA fragments are separated within the gel.

3. It is estimated that less than 1% of rapes lead to felony convictions. List a few reasons why this might be the case.

4. Rape is a major issue facing women that is just now being brought more into the public with the #Metoo movement. Go to the website below and research the statistics below.

   https://www.rainn.org/statistics/victims-sexual-violence

   a. What age group is most commonly affected by rape?

   b. What percentage of women will be the victim of rape in their lifetime?

   c. What are some of the long-term effects on victims of rape?

5. Explain the function of each

   a. Gel

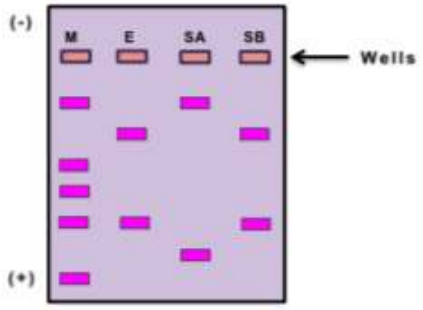
   b. Restriction enzymes

   c. Electric current

   d. VNTR

   e. Radioactive probes

6. DNA profiles from two suspects (SA and SB) are shown below along with evidence (E) found at a murder scene (a blood drop). The marker (M) is shown for reference of sizes. Based on the results shown, which of the two will be most likely exonerated in this case? Explain your conclusion.



7. Write the complementary base pairs to the strand of DNA below

   A - G - C - T - T - C - G - A - T - A - G - C - C - T - A - C - A - T - C - A - G - C - T - A

Standards

5'AGCTTTTA3'

5'AGCTTCATTTA3'

5'AGCTTCATCATTTA3'

5'AGCTTCATCATCATTTA3'

5'AGCTTCATCATCATCATTTA3'

5'AGCTTCATCATCATCATCATTTA3'

5'AGCTTCATCATCATCATCATCATTTA3'

5'AGCTTCATCATCATCATCATCATCATTTA3'

Victim

5'CTAGAAGCTTAAAGCTTCATCATTTAAGCTTCAAAGCTTTCGACCTAAATTGC3'

5'CTAGAAGCTTAAAGCTTCATCATCATCATCATTTAAGCTTCAAAGCTTTCGAC3'

Suspect #1

5'CTAGAAGCTTAAAGCTTCATCATCATTTAAGCTTCAAAGCTTTCGACCTAAAT3'

5'CTAGAAGCTTAAAGCTTCATCATCATCATCATCATCATTTAAGCTTCAAAGCTT3'

Suspect #2

5'CTAGAAGCTTAAAGCTTCATCATCATCATCATCATTTAAGCTTCAAAGCTTTCG3'

5'CTAGAAGCTTAAAGCTTCATCATCATCATTTAAGCTTCAAAGCTTTCGACCTA3'

Crime Scene DNA (Pulled from victim)

5'CTAGAAGCTTAAAGCTTCATCATTTAAGCTTCAAAGCTTTCGACCTAAATTGC3'

5'CTAGAAGCTTAAAGCTTCATCATCATCATCATCATCATTTAAGCTTCAAAGCTT3'